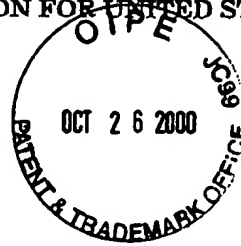


08/198,037

APPLICATION FOR UNITED STATES PATENT



RECEIVED

NOV 02 2000

TECH CENTER 1600/2900

Inventor(s): Jürgen ENGEL
Burkhard WICHERT
Dieter SAUERBIER
Thomas REISSMANN

Invention: NOVEL PROCESS FOR THE PREPARATION
OF CETRORELIX LYOPHILIZATE

Cushman, Darby & Cushman
1100 New York Avenue N.W.
Washington, D.C. 20005-3918

Attorneys

Telephone: (202) 861-3000

SPECIFICATION



RECEIVED

NOV 02 2000

NOVEL PROCESS FOR THE PREPARATION OF
CETRORELIX LYOPHILIZATE

TECH CENTER 1600/2900

The present invention relates to the preparation of a lyophilizate of a peptide and the use of the lyophilizate in the treatment of infertility and to provide male gonad protection.

BACKGROUND OF THE INVENTION

Cetrorelix is a decapeptide with a terminal acid amide group that is used in the form of its acetate salt. The synthesis and some pharmacological effects are described in European patent application 299 402 (U.S. Patent 4,800,191).

It should be possible to administer the active substance subcutaneously in a dose of 0.1 to 20 mg. Aqueous solutions of the decapeptide are unstable, and, therefore, autoclaving in the container used to distribute it is not possible. During conventional sterilization, using the prescribed conditions, the decapeptide tends to decompose. To obtain an injectable solution it was therefore necessary to develop a lyophilizate.

The amount of active substance in the solution to be lyophilized is, however, so small that, in low active substance concentrations, only a loose fluff results on the glass wall of the ampoule after drying the solution free of auxiliary substances, and this fluff is carried out of the vial with the stream of ~~steam used for sterilization purposes~~ ^{water vapor generated by the sublimation process}. It is therefore necessary to use a bulking agent that forms a stable cake. In high concentrations, this auxiliary substance can be dispensed with. The following auxiliary substances may be considered as bulking agents: hexitols, in particular mannitol, glucitol, sorbitol, such as D-sorbitol, dulcitol, allitol, altritol (for example D- and L-altritol), iditol (for example D- and L-iditol), their optically active forms (D- and L-forms) as well as the

Widat 10.2.94
Ludwig 10.2.94
Bo-71 15.2.94

Lu 2/8 94 - 1 -

corresponding racemates. Mannitol is used in particular, such as D-mannitol, L-mannitol, DL-mannitol, sorbitol and/or dulcitol, and, of these, D-mannitol is preferred. The hexitol used may also be composed of a mixture of the hexi-
5 tols named, for example a mixture of mannitol and sorbitol and/or dulcitol. Since dulcitol is less water soluble than, for example, mannitol, the dulcitol content in the aqueous solution should not exceed for example 3 percent by weight. Mannitol and sorbitol, on the other hand, can for example be
10 mixed in any ratio.

Apart from hexitol it is also possible to add other, conventional pharmaceutical auxiliary substances, such as amino acids, such as alanine, glycine, lysine, phenylalanine, asparaginic acid, glutaminic acid, leucine, lactose,
15 polyvinylpyrrolidone, glucose, fructose, albumin and equivalent bulking agents. Urea and sodium chloride may also be used as bulking agents. The total amount of such substances in the solution which is used for freeze-drying, is for example 0 - 16.9 parts by weight, for example 0.1 - 7
20 parts by weight, based on 1 part by weight of cetrorelix. In the finished lyophilizate the total amount of such auxiliary substances may be up to 16.9 parts by weight, based on one part by weight of hexitol. In detail, the amount of such auxiliary substances depends on the amount of
25 hexitol present and to such an extent that the total amount of hexitol and such other auxiliary substances in the finished lyophilizate may not be more than a maximum of 17 parts by weight, based on 1 part by weight of cetrorelix. If only 0.1 part by weight of hexitol is present in the ly-
30 ophilizate, it is thus possible to have up to 16.9 parts by weight of other auxiliary substances; if, for example, 8.5 parts by weight of hexitol are present, the amount of other

auxiliary substances may for example be up to 8.5 parts by weight, based on 1 part by weight of cetrorelix.

It was, however, found, during development work on the lyophilizate, that the active substance behaves in a widely variable and unpredictable manner during processing. The first batches gave good results, but it soon transpired that difficulties occurred during sterile filtration and faulty batches resulted.

It is known from the literature, for example from Powell, M.F.; Pharmaceutical Research, 1258-1263 (8)1991; Dathe, M: Int. J. Peptide Protein Res. 344-349 (36) 1990; Szejtli, J.: Pharmaceutical Technology International 16-22, 1991 that oligopeptides, particularly those with terminal acid amide function, tend to form gels. During sterile filtration this is apparent from the speed of filtration, indeed, the increased viscosity of such solutions can often already be detected organoleptically. A gelatinous layer remains on the sterile filter. It is then no longer possible to prepare a medication with an exactly and reproducibly defined active substance content.

Table 1 lists various results of the first 11 batches.

The active substance contents fluctuate between 100% and 36%.

Table 1: Cetrorelix acetate

Batch	Dosage	Active substance content %
-------	--------	-------------------------------

1	100 µg	100
2	500 µg	100
3	500 µg	90
4	500 µg	36
5	500 µg	100

	Batch	Dosage	Active substance content
			%
	6	500 µg	85
5	7	1 mg	80
	8	1 mg	100
	9	2 mg	100
	10	2 mg	80
10	11	2 mg	100

To avoid this gel formation, the literature lists the following additives which may be tried out on an experimental basis:

Organic solvents may be considered, for example
15 acetonitrile, n-butanol, tertiary butanol, ethanol, isopropanol, octanol and benzyl alcohol. It is also possible to use salts and buffer solutions, such as acetate buffer, citrate buffer, sodium chloride, sodium phosphate, sodium EDTA, sodium bicarbonate, phosphate buffer, guanidine acetate, urea.
20

Polymers may also be used, such as gelatin, polyethylene glycol 600, hydroxyethyl starch, polyvinylpyrrolidone, polyvinyl alcohol. The use of amino acids, for example alanine, glycine, lysine, phenylalanine, asparaginic acid, glutaminic acid and leucine has also been described.
25 Acids that were used were citric acid, caprylic acid, octanoic acid, hydrochloric acid, sulphuric acid and acetic acid. Physiologically acceptable surfactants that may be used are benzalkonium chloride, cetyl alcohol, bile acids, lecithins, polysorbates, Spans® and Pluronic®.
30

Carbohydrates and cyclodextrins such as glucose, lactose, mannitol, saccharose, alpha-, beta- and gamma

cyclodextrins, hydroxypropyl-alpha- and beta-cyclodextrins, hydroxyethyl cyclodextrins and methyl cyclodextrins have already been used. These auxiliary substances were tested as filtration supporting agents to prevent gel formation.

5 No satisfactory solution of the problem could, however, be found. Only acidification with acetic acid showed partial success. Here, too, it was, however, always necessary to accept high filtration losses.

SUMMARY OF THE INVENTION

10 It was then surprisingly found that cetorelix can be easily dissolved in 30% volume/volume acetic acid. The solution is then diluted to a final concentration of 3% cetorelix with water for injection purposes and mannitol is added. Although it is stated in the literature that the
15 terminal amide group hydrolyzes easily in acid medium, this was not found in the case of cetorelix. Solutions prepared according to this method caused no difficulties during filtration. The correct amounts of active substance were always found.

20 Thus, in accordance with the present invention, a peptide which contains 3-15 amino acids is dissolved in acetic acid to form a solution containing 100 - 10,000 parts by weight of acetic acid for each part of peptide, the solution is transferred to water, and the resulting solution is
25 lyophilized.

The filtration speed of the acetic acid solution attains values that ensure satisfactory production sequences. A general process for sterile lyophilization is described in pages 557 - 559 of Sucker, Fuchs and Speiser (Publishers)
30 "Pharmazeutische Technologie" 2nd edition 1991, Thieme-Verlag, Stuttgart-New York. A further description of the lyophilization process used is given in German published specification (DOS) 37 35 614 (U.S. Patent 5,204,335).

The lyophilizate is used in the treatment of female sterility. One therapeutic process has hitherto consisted in stimulating follicle maturation using human menopause gonadotrophin and then triggering ovulation by administering 5 human chorion gonadotrophin. The ovulation triggered thereby occurred 32 hours later. The resulting ova are available for in vitro fertilization.

A disadvantage of this treatment ~~with agonists~~ is the fact that up to 10 follicles mature during the stimulation 10 phase. This elevated follicle maturation leads to hormone level peaks in the LH. These peaks result in an early stage of follicle maturation and ovulation at an unpredicted point in time. This impaired ovulation occurs in about 25% of treated cases and is a disadvantage since the cycle that 15 displays disturbed ovulation of this kind cannot be used for the collection of ova and the entire treatment has to be repeated about 1 month later.

Another disadvantage of the conventional simulation ~~and the use of GnRH-agonists in order to avoid premature LH-peaks,~~ treatment ~~is~~ the long treatment duration of 4 weeks which is 20 needed to achieve satisfactory suppression. The agonists continue to display a hyperstimulation syndrome in 1-2% of cases in which the follicle cells hypertrophy. The risk of hyperstimulation is particularly great in the case of polycystic ovaries. The hyperstimulation syndrome is a severe 25 side effect which can lead to fatalities.

It has now been found that the antagonist cetrorelix displays the following advantages in this treatment:

Treatment with cetrorelix over 5 days is sufficient to achieve total suppression. ~~The hyperstimulation syndrome~~ ^{premature LH-peaks} ~~and the frequency of hyperstimulation syndrome should be reduced~~ 30 cannot arise. In addition, less HMG is used in the 2nd phase of therapy, the ovulation triggering phase. This gives this in-vitro fertilization treatment a not inconsiderable cost advantage. In-vitro fertilization is, for

15.2.94 Ricks

Michael 21.2.94

4/11/94
Stark 2/23/94

example, used when a tube anomaly is present. To perform this treatment it is necessary to precisely monitor the cycle and to establish the time of ovulation as precisely as possible. This has hitherto only been achieved to a limited extent since preovulatory LH increase often occurred too early due to stimulation with HMG/HCG, or was not maintained for a sufficiently long period. Avoidance of this premature increase is, however, of critical importance for the success of the treatment in order to precisely determine the time of fertilization. This reduces the physical and mental burden on the patient and makes optimum use of hospital logistics. To achieve this objective with great reliability it is necessary to suppress endogenous hormone production (LH-FSH, oestradiol) as completely as possible in order to simultaneously stimulate follicle maturation through administration of exogenous gonadotrophins (HMG/HCG) and to monitor the hormone status at any time. It is only when a sufficiently large number of follicles have been achieved (4-6), having approximately the same degree of maturation, that ovulation is triggered by administering an HCG bolus injection.

Use of an antagonist makes treatment substantially more successful and safer for the patient.

Another area of use of the cetorelix lyophilizate according to the present invention is to protect the gonads in male patients. Male patients are pre-treated with cetorelix lyophilizate and the activity of the gonads is reinforced. As a result, other harmful noxious agents, such as radiation therapy or treatment with cytostatics, have no or only a small possibility of affecting the sensitive tissue of the gonads.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The following Example illustrates the invention.

Example of the method of preparation:

Approx. 1.5 liters of water for injection purposes are prepared in a suitable glass vessel. 210 g water for injection purposes are prepared in another glass vessel and 91.17 g acetic acid are added. The amount of cetorelix acetate calculated (1.62 - 1.695 g, depending on the content of the batch used) is dissolved in the prepared 30% acetic acid with stirring. This solution is transferred to the glass vessel with 1.5 liters of water for injection purposes, 82.2 g mannitol are added, dissolved and made up to 3039 g with water for injection purposes.

In-process checks:

pH value: 2.5 - 3.0
Density: 1.009 - 1.017 g/cm³ at 20°C

Refractive index:

1.227 - 1.340 at 440 nm and 20°C

The solution is sterilized by filtration through an appropriate membrane filter (pore size 0.2 µm) under aseptic conditions. 100 ml first runnings should be discarded. The filters should be sterilized with superheated steam *before sterile filtration*. Cetorelix freeze-dried solution should be protected from recontamination during storage.

The solution is immediately filled into colorless injection bottles DIN 2R, hydrolytic class I under aseptic conditions and provided with sterile freeze-drying stoppers. The nominal filling amount is 2.0 ml = 2.026 g.

The 2 ml injection bottles were rinsed in an injection bottle washing machine, dried with hot air and sterilized. The cleaned, freeze-drying stoppers were autoclaved. The closed injection bottles were transferred to a freeze-drying installation and frozen at a plate temperature of -40°C.

Wickert 10.2.94
Jewell 10.2.94
Wess 15.02.94



2/8/94

- 8 -

Drying was carried out using a drying program with a plate temperature of -40°C rising to $+20^{\circ}\text{C}$. The installation is then flooded with sterile nitrogen, the bottles are closed in the installation and the stoppers secured with crimped 5 caps.

The injection bottles are checked visually for faulty closures and outer faults. Faulty injection bottles are removed and destroyed.

Cetrorelix lyophilizate 1 mg is a white, solid, freeze-dried cake in a colorless 2 ml injection bottle which is closed with gray freeze-drying stoppers and yellow flip-off crimped caps.

15

20

25

30